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TOXICOLOGY OF 1,1,2-TRICHLOROETHANE IN THE MOUSE

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ABSTRACT

1,1,2-Trichloroethane (TCE) was administered to male and female CD-1 mice to evaluate its effect on standard toxicological parameters. Following determination of the acute LD50 (378 mg/kg in males and 491 mg/kg in females), and a 14-day range-finding study, a 90-day drinking water study was performed in which the doses consumed were 4.4, 46, and 305 mg/kg for males and 3.9, 44, and 384 mg/kg for females. The liver was a target of TCE toxicity in both sexes as demonstrated by dose-dependent alterations in hepatic microsomal enzyme activities and serum enzyme levels. The erythroid element of the female mice was also affected, as indicated by significantly decreased hematocrit and hemoglobin levels.

INTRODUCTION

1,1,2-Trichloroethane (TCE) is one of the organic chemicals detected in drinking water supplies at levels from 0.1 to 8.5 µg/l[1]. It also has been detected in industrial effluents at levels of 5.4 mg/l[2]. TCE is used as a solvent for fats, oils, waxes, and resins and in the synthesis of organic chemicals[3].

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Also, in water treatment, small amounts of TCE can be formed during chlorination[3].

All of the toxicological data available on TCE are derived from short-term exposure (e.g., a single i.p. injection), except for the gavage studies performed by the NCI for carcinogenicity bioassays[4]. Acute oral toxicity studies on TCE using both male and female mice have not been reported. Klassen and Plaa[5] reported that the i.p. LD50 of TCE in male mice is 242 mg/kg. Smyth et al.[6] reported the oral LD50 in rats to be 580 mg/kg and the dermal LD50 in rabbits to be 3730 mg/kg.

Other toxicological studies on TCE evaluated the hepatic and renal effects following a single i.p. injection. Hepatotoxicity and nephrotoxicity have been investigated primarily in mice and dogs. Cellular infiltration, hepatocyte vacuolation, elevation of SGPT, and prolonged bromosulfophthalein retention have been observed in mice[5]. Increases in SGPT levels in mice treated i.p. with TCE were even greater when the mice receive isopropyl alcohol or acetone[7]. In dogs, mild centrilobular necrosis, slight subcapsular necrosis, and vacuolization of centrilobular hepatocytes have been observed in association with increases in SGPT levels[8]. In experiments designed to examine nephrotoxicity, the presence of hyaline droplets, nuclear pyknosis, hydropic degeneration, increased eosinophilia, tubular necrosis with karyolysis, and loss of the epithelium of convoluted tubules have been reported in mice in association with altered phenolsulfonphthalein (PSP) excretion[5]. In dogs, tubular necrosis and decreased PSP excretion have been observed after i.p. injection of TCE[8].

The toxicological investigations presented in this manuscript were undertaken to provide data that would assist in the hazard assessment of this chemical in the drinking water. After the LD50 was determined in mice by the oral route, a 14-day range-finding study was performed to provide information neces-

sary for designing a 90-day study in which the compound was administered in the drinking water. This manuscript reports the general toxicological status of mice exposed orally to TCE, while the following manuscript will discuss the immune status of these mice in relation to the toxicological findings[9].

MATERIALS AND METHODS

Details of the methods for the general toxicological assays and historical controls have been fully described by White et al. in the first article of this volume[10].

Chemical

1,1,2-Trichloroethane (TCE), lots 070177 and 061197, was purchased from Aldrich Chemical Co. (Milwaukee, WI). The purity was given as 95%. The structure was verified by infrared spectrometry and the purity verified by gas chromatography. No stabilizers or preservatives were present with this chemical.

Chemical Administration

Solutions of TCE were prepared fresh daily for the acute and 14-day studies and the appropriate concentrations were administered by gavage in a volume of 0.01 ml/g of body weight to achieve the desired dose. Solutions were prepared on the day of administration in a 1:9 (v/v) solution of emulphor (GAF Corp., New York, NY) and deionized water, and maintained in dark glassware at 4°C until used. In the acute study, TCE was administered as a single dose to male and female mice after an 18 hr fast, at seven doses from 200 to 600 mg/kg. In the 14-day study, concentrations of TCE were prepared so that each mouse received 1/100 and 1/10 the LD50 daily for 14 days (3.8 and 38 mg/kg). For the 90-day study, TCE was diluted in deionized water to achieve con-

centrations of 0.02, 0.2, and 2.0 mg/ml and administered in the drinking water. These concentrations resulted in a delivery of approximately 1/100, 1/10 and 1/1 the LD50. The solutions were maintained at room temperature in amber-colored bottles with stainless steel spouts fitted through cork stoppers. Water bottles were changed twice weekly. Less than 10% of the TCE was lost during the three or four days between bottle changes, as measured by GLC with head space analysis.

Fluid consumption during the 90-day study was estimated by the change in fluid weight over the three or four day period when solutions were changed. Twelve cages of control mice and eight cages of each treatment group for each sex were used to estimate fluid consumption. Chemical exposure was calculated from fluid consumption and is reported as mg TCE/kg/day. Fluid consumption is reported as ml/kg/day.

Statistical Evaluation

If a one-way analysis of variance of the means showed treatment effects, a Duncan's Multiple Range Test was performed[11]. Values which differ from vehicle control at $p < 0.05$ were considered statistically significant. Each of the values represents the mean ± SE.

RESULTS

Acute Toxicity

The acute toxicity of TCE was performed to determine doses for use in the 14-day range-finding studies and to define a toxicological profile following exposure by the gastrointestinal route. The LD50's for TCE administered as a single gavage to male and female mice were 378 and 491 mg/kg, respectively (Table 1). All mice receiving doses greater than or equal to 450 mg/kg

TABLE 1
Acute Toxicity (mg/kg) of TCE in CD-1 Mice[a]

Sex	Number of Mice	LD50	Log Probit Slope
Male	56	378 (344 - 408)	17.6
Female	56	491 (452 - 550)	13.7

[a]CD-1 male and female mice were fasted for 18 hr prior to a single gavage of TCE. The LD50 with corresponding 95% confidence limits (numbers in parentheses) and the slope were calculated according to the Log Probit Procedure[12].

became sedated within an hour, while 10% of these mice lost their righting reflex. At the end of the fourth hour, most animals had recovered from the anesthetic effect of TCE and did not appear to be in acute distress. Most deaths occurred within 24 hr, and no deaths occurred after 48 hr. Observations following chemical administration were performed over a 14-day period. At the time of death, necropsies revealed a dose-dependent irritation of the upper gastrointestinal tract, with 100% of the mice at the 500 and 600 mg/kg dose levels showing gastric irritation. Fifty to 75% of the animals in each group had pale livers, and up to 25% demonstrated lung damage as manifested be reddened or hemorrhagic areas.

Fourteen Day Study

No significant differences were observed between the body weights of TCE-exposed mice and control mice over the 14-day gavage period, and no exposure-related mortality occurred (data not shown). Brain, thymic, and testicular weights increased significantly in male mice dosed at 38 mg/kg when expressed on

TABLE 2
Body and Organ Weights of Male CD-1 Mice Exposed by Gavage for Fourteen Days to TCE[a]

Exposure Group	Body Wt. (g)	Organ	Wt. (mg)	Percent Body Wt.	Organ/ Brain
Vehicle[b]	27.9 ± 1.1	Brain	408 ± 15	1.48 ± 0.06	-
		Liver	1699 ± 92	6.09 ± 0.23	4.21 ± 0.24
		Spleen	158 ± 14	0.56 ± 0.04	0.39 ± 0.03
		Lungs	202 ± 14	0.71 ± 0.03	0.48 ± 0.03
		Thymus	67 ± 4	0.24 ± 0.02	0.16 ± 0.01
		Kidneys	458 ± 33	1.62 ± 0.07	1.11 ± 0.05
		Testes	194 ± 9	0.70 ± 0.03	0.48 ± 0.02
3.8 mg/kg	28.1 ± 0.6	Brain	414 ± 10	1.48 ± 0.04	-
		Liver	1870 ± 68	6.67 ± 0.22*	4.52 ± 0.12
		Spleen	145 ± 7	0.52 ± 0.03	0.35 ± 0.02
		Lungs	199 ± 10	0.71 ± 0.03	0.48 ± 0.02
		Thymus	70 ± 6	0.25 ± 0.02	0.17 ± 0.01
		Kidneys	460 ± 21	1.63 ± 0.06	1.11 ± 0.04
		Testes	190 ± 10	0.68 ± 0.04	0.46 ± 0.02
38 mg/kg	29.8 ± 0.6	Brain	453 ± 9*	1.52 ± 0.03	-
		Liver	1917 ± 53	6.43 ± 0.09	4.52 ± 0.12
		Spleen	154 ± 8	0.52 ± 0.02	0.34 ± 0.02
		Lungs	225 ± 8	0.75 ± 0.02	0.50 ± 0.02
		Thymus	84 ± 4*	0.28 ± 0.01	0.19 ± 0.01
		Kidneys	489 ± 16	1.64 ± 0.04	1.08 ± 0.03
		Testes	228 ± 8*	0.76 ± 0.02	0.50 ± 0.02

[a]Values represent mean ± SE derived from 12 mice per group.
[b]Vehicle = 10% emulphor
*Significantly different from vehicle at $p < 0.05$.

a total organ weight basis (Table 2). When expressed as a percent of body weight, or as an organ to brain ratio, no significant changes occurred. Hematological, coagulation, SGPT, and BUN values were all within the range of vehicle control values (Table 3). Lactic dehydrogenase activity was depressed 21% below control in the 38 mg/kg group.

TABLE 3
Selected Hematological, Coagulation, and Serum Chemistry Values for Male CD-1 Mice Exposed by Gavage for 14 Days to TCE[a]

Parameter	Vehicle[b]	3.8 mg/kg TCE	38 mg/kg TCE
Hematocrit (%)	41.4 ± 0.7	41.9 ± 0.6	41.4 ± 0.7
Hemoglobin (g%)	11.8 ± 0.4	11.6 ± 0.4	13.1 ± 0.5
Leukocytes ($10^3/mm^3$)	8.25 ± 0.68	7.31 ± 0.61	7.47 ± 0.60
Fibrinogen (mg%)	283 ± 7	280 ± 7	275 ± 9
Prothrombin Time (sec)	8.3 ± 0.1	8.3 ± 0.1	8.3 ± 0.1
LDH[c] (IU/L)	1059 ± 47	954 ± 55	835 ± 62*
SGPT[c] (IU/L)	88.3 ± 9.5	82.8 ± 12.9	59.2 ± 5.0
BUN[c] (mg%)	27.3 ± 1.0	26.6 ± 1.6	25.2 ± 0.9

[a]Values represent mean ± SE derived from 11-12 mice per group.
[b]Vehicle = 10% emulphor
[c]LDH = lactate dehydrogenase; SGPT = serum glutamic-pyruvic transaminase; BUN = blood urea nitrogen.
*Significantly different from vehicle at $p < 0.05$.

Ninety Day Study

On the foundation of the data from the acute toxicity and 14-day range-finding studies, concentrations of TCE in the drinking water solutions were designed to deliver daily doses equivalent to, and higher than, those administered by gavage. The desired doses were 3.8, 38, and 380 mg/kg (1/100, 1/10 and 1/1 the LD50) and the concentrations of chemical calculated to deliver these doses in the drinking water were 0.02, 0.2, and 2.0 mg/ml. The 380 mg/kg dose was included as the data from the 14-day exposure indicated that higher doses may be tolerated. The calculated time-weighted average doses delivered based on fluid consumption and body weights were 4.4, 46, and 305 mg/kg for the male mice and 3.9, 44, and 384 mg/kg for the female mice (Table 4). Monthly time-weighted averages for fluid consumption are also shown in Table 4. On a ml/kg basis, there was a 30% decrease in fluid consumption in male mice drinking the highest

TABLE 4
Time-Weighted Averages of Consumption of Fluid by CD-1 Mice Receiving TCE in the Drinking Water for 90 Days[a]

Concentration (mg/ml)	Days of Study[b] 0-30	31-60	61-90	Cumulative (Days 0-90) ml/kg/day	mg/kg/day[c]	ml/mouse day[d]
			MALES			
0	252 ± 5	242 ± 4	225 ± 4	240 ± 5	0	8.2 ± 0.1
0.02	203 ± 7	241 ± 5	209 ± 5	219 ± 4	4.4 ± 0.1	7.8 ± 0.1
0.20	226 ± 5	254 ± 7	207 ± 5	228 ± 6	46 ± 1.2	7.6 ± 0.2
2.00	162 ± 4*	152 ± 5*	139 ± 6*	153 ± 9*	305 ± 18.2	4.5 ± 0.2*
			FEMALES			
0	230 ± 5	171 ± 4	172 ± 4	193 ± 3	0	5.2 ± 0.1
0.02	192 ± 6	198 ± 5	197 ± 4	195 ± 5	3.9 ± 0.1	5.3 ± 0.1
0.20	206 ± 6	265 ± 6	197 ± 4	222 ± 7	44 ± 1.3	6.0 ± 0.1
2.00	184 ± 4	212 ± 5	186 ± 5	192 ± 5	384 ± 9.5	5.3 ± 0.1

[a]Values represent mean ± SE derived from 48 mice in the control (deionized water) group and 32 mice in the other groups. Means were calculated for each month of the study, as well as for the entire 90 days.
[b]Average ml fluid/kg body weight/day for the period indicated.
[c]Average mg of TCE/kg body weight/day.
[d]Average ml fluid/mouse/day.
*Significantly different from control at $p < 0.05$.

concentration of TCE over the 90-day period. However, there was no TCE-related change in fluid consumption for the female mice.

The growth curves for mice exposed to TCE reflect their fluid consumption pattern. Male mice demonstrated a concentration-dependent reduction in weight gain (Figure 1). Although the animals were randomized at the beginning of exposure and an analysis of variance among the cages showed no difference in body weights, there was an early (day 3) reduction in weight for the male mice receiving the highest concentration of TCE. After this weight loss, these mice resumed a normal growth pattern,

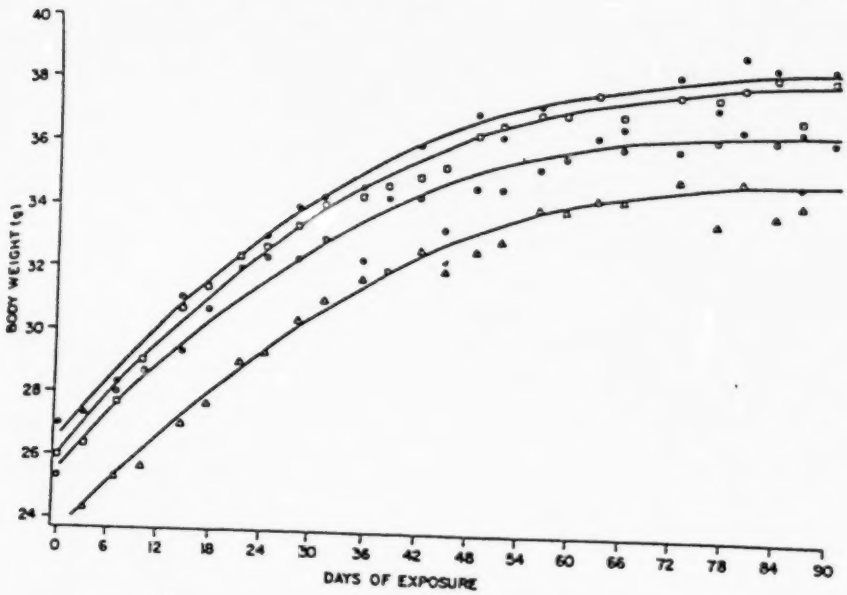

FIG. 1. Growth chart of male CD-1 mice exposed to TCE in the drinking water: • deionized water; □ 4.4 mg/kg delivered, 0.02 mg/ml in water; o 46 mg/kg delivered, 0.2 mg/ml in water; ▵ 305 mg/kg delivered, 2.0 mg/ml in water. There were 48 mice in the deionized water group, and 32 mice in each of the TCE-exposed groups.

although they always lagged behind the other mice due to the initial reduction. Over the 90-day exposure period, male mice exposed to deionized water gained 12.2 ± 0.5 g (mean ± SE), while the male mice exposed to 0.02 and 0.2 mg/ml TCE gained 11.8 ± 0.5 and 11.2 ± 0.6 g, respectively. Male mice exposed to 2.0 mg/ml gained 7.9 ± 1.1 g, which was significantly less than the control group. In contrast to the males, the weight gain of female mice exposed to TCE was not altered from control (Figure 2).

Organ weights for male and female mice are shown in Tables 5 and 6. In males, there were no significant changes in weights for the brain, spleen, lungs, and thymus. On a mg basis, liver

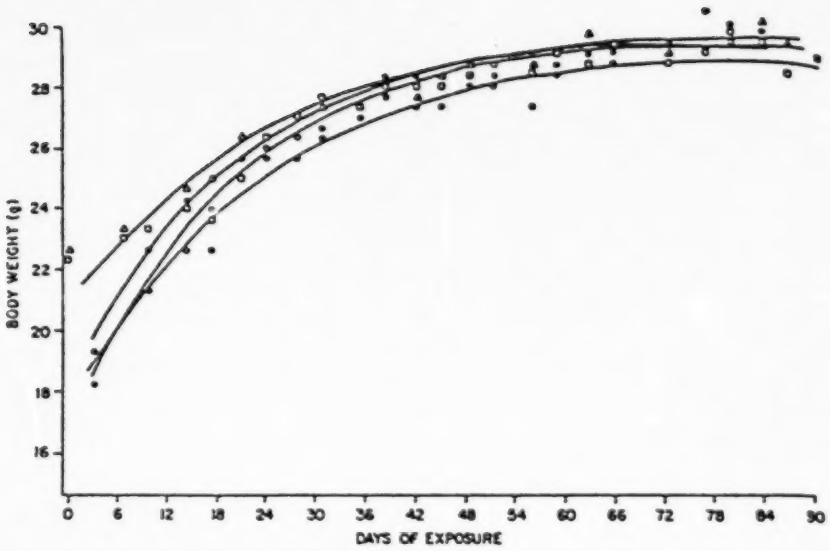

FIG. 2. Growth chart of female CD-1 mice exposed to TCE in the drinking water: • deionized water; □ 3.9 mg/kg delivered, 0.02 mg/ml in water; o 44 mg/kg delivered, 0.2 mg/ml in water; ▲ 384 mg/kg delivered, 2.0 mg/ml in water. There were 48 mice in the deionized water group, and 32 mice in each of the TCE-exposed groups.

weights in male mice exposed to 0.2 and 2.0 mg/ml TCE were 11 and 12% smaller than the control group. This decreased liver weight was proportional to the decreased body weight; therefore, no significant changes could be measured on a percent of body weight basis. Similarly, kidneys weights were decreased 12 and 16% on a mg basis for the groups receiving 0.2 and 2.0 mg/ml. Again, no change from control was evident on a percent of body weight basis. Testicular weights of males exposed to the two highest concentrations increased 11 and 12% above control, but only on a percent of body weight basis. Females responded differently in that the group receiving 2.0 mg/ml TCE had a 32% increase in absolute liver weight and a 26% increase when expressed as per-

TABLE 5
Body and Organ Weights of Male CD-1 Mice Exposed to TCE in the Drinking Water for 90 Days[a]

Parameter	Deionized Water	TCE 0.02 mg/ml	TCE 0.2 mg/ml	TCE 2.0 mg/ml
Body Wt. (g)	39.7 ± 0.7	41.0 ± 0.5	37.6 ± 1.1	35.8 ± 1.0*
Brain (mg)	490 ± 7	498 ± 8	480 ± 11	468 ± 14
(% body wt.)	(1.24)	(1.22)	(1.29)	(1.31)
Liver (mg)	2125 ± 57	2152 ± 58	1889 ± 90*	1875 ± 45*
(% body wt.)	(5.33)	(5.24)	(5.01)	(5.25)
Spleen (mg)	190 ± 25	175 ± 13	152 ± 13	137 ± 8
(% body wt.)	(0.48)	(0.42)	(0.41)	(0.38)
Lungs (mg)	239 ± 6	237 ± 7	241 ± 12	228 ± 5
(% body wt.)	(0.60)	(0.58)	(0.64)	(0.64)
Thymus (mg)	39 ± 2	39 ± 3	45 ± 4	42 ± 4
(% body wt.)	(0.10)	(0.09)	(0.12)	(0.12)
Kidneys (mg)	660 ± 17	648 ± 24	580 ± 13*	555 ± 22*
(% body wt.)	(1.66)	(1.58)	(1.55)	(1.55)
Testes (mg)	255 ± 6	259 ± 6	263 ± 8	258 ± 7
(% body wt.)	(0.64)	(0.63)	(0.71)*	(0.72)*

[a]Values represent mean ± SE derived from 23 mice in the deionized water group and 14-16 mice in the other groups.
*Significantly different from control at $p < 0.05$.

cent of body weight. Spleen and kidney weights of females exposed to the highest concentration were significantly increased on a mg basis only.

There were few TCE exposure-related alterations in the hematological or blood coagulation parameters in the male mice (Tables 7 and 8). Male mice exposed to the highest concentration of TCE had decreased percent of polymorphonuclear leukocytes and

TABLE 6
Body and Organ Weights of Female CD-1 Mice Exposed to TCE in the Drinking Water for 90 Days[a]

Parameter	Deionized Water	TCE 0.02 mg/ml	TCE 0.2 mg/ml	TCE 2.0 mg/ml
Body Wt. (g)	29.9 ± 0.4	29.8 ± 0.6	30.0 ± 0.8	31.2 ± 0.6
Brain (mg)	528 ± 4	521 ± 6	522 ± 9	516 ± 6
(% body wt.)	(1.77)	(1.76)	(1.75)	(1.66)*
Liver (mg)	1368 ± 41	1410 ± 32	1318 ± 54	1806 ± 82*
(% body wt.)	(4.57)	(4.74)	(4.39)	(5.76)*
Spleen (mg)	132 ± 6	134 ± 4	117 ± 8	156 ± 12*
(% body wt.)	(0.44)	(0.45)	(0.38)	(0.49)
Lungs (mg)	213 ± 5	220 ± 8	215 ± 8	225 ± 6
(% body wt.)	(0.71)	(0.74)	(0.72)	(0.72)
Thymus (mg)	49 ± 2	42 ± 3	41 ± 3	50 ± 3
(% body wt.)	(0.16)	(0.14)	(0.14)*	(0.16)
Kidneys (mg)	392 ± 9	386 ± 15	380 ± 13	430 ± 10*
(% body wt.)	(1.31)	(1.30)	(1.27)	(1.38)

[a]Values represent mean ± SE derived from 23 mice in the deionized water group and 16 mice in the other groups.
*Significantly different from control at $p < 0.05$.

increased percent of lymphocytes. This shift was probably due to a minor biting problem among the male mice in the control and low dose groups, which resulted in a lower than normal percent of lymphocytes and a higher than normal percent of polymorphonuclear cells in these groups.

Hemoglobin and hematocrit values of female mice exposed to 2.0 mg/ml TCE decreased 6 and 5% ($p < 0.05$), as compared to the mice receiving deionized water. Erythrocytes also decreased, but not at the $p < 0.05$ level (Table 9). Platelet counts increased in

TABLE 7
Hematology of Male CD-1 Mice Exposed to TCE in the Drinking Water for 90 Days[a]

Parameter	Deionized Water	TCE 0.02 mg/ml	TCE 0.2 mg/ml	TCE 2.0 mg/ml
Hematocrit (%)	39.8 ± 0.9	40.1 ± 1.3	40.3 ± 0.6	38.6 ± 0.9
Hemoglobin (g%)	13.8 ± 0.4	13.5 ± 0.4	13.4 ± 0.3	13.2 ± 0.3
Erythrocytes ($10^6/mm^3$)	7.70 ± 0.24	7.71 ± 0.35	7.96 ± 0.21	7.01 ± 0.19
Leukocytes ($10^3/mm^3$)	6.79 ± 0.55	5.63 ± 0.48	5.42 ± 0.44	6.57 ± 0.66
Platelets ($10^5/mm^3$)	4.08 ± 0.17	3.85 ± 0.29	3.80 ± 0.22	4.05 ± 0.28
Fibrinogen (mg%)	292 ± 16	265 ± 20	273 ± 17	262 ± 14
Prothrombin Time (seconds)	9.2 ± 0.1	9.3 ± 0.2	9.4 ± 0.1	9.3 ± 0.1
APTT[b] (seconds)	31.8 ± 1.5	30.8 ± 0.9	29.0 ± 1.1	30.8 ± 0.9

[a]Values represent mean ± SE derived from 23 mice in the deionized water group and 14-16 mice in the other groups.
[b]APTT = activated partial thromboplastin time.

all groups, but not in a dose-dependent fashion. A 48% increase in leukocyte number was also observed in the females. However, the biological significance of this is questionable, primarily because the leukocyte count of the deionized water control group is at the low end of the historical control data accumulated in our laboratory (6.37 ± 2.94)[10]. Fibrinogen levels increased in females exposed to all three exposure levels, while prothrombin times decreased in the two highest exposure groups.

TABLE 8
Differential Cell Counts of CD-1 Mice Exposed to TCE in the Drinking Water for 90 Days[a]

Exposure Group	Percent of Total White Blood Cells			
	Lymphocytes	Polymorphonuclears	Monocytes	Eosinophils
		MALES		
Deionized Water	53.3 ± 2.9	40.4 ± 2.9	5.1 ± 0.6	1.2 ± 0.3
0.02 mg/ml	58.2 ± 4.2	37.2 ± 4.2	3.6 ± 0.7	0.9 ± 0.4
0.2 mg/ml	61.8 ± 3.8	34.3 ± 3.3	2.9 ± 0.8	1.1 ± 0.3
2.0 mg/ml	72.2 ± 1.8*	22.4 ± 1.6*	4.2 ± 0.7	1.2 ± 0.4
		FEMALES		
Deionized Water	81.0 ± 1.6	13.1 ± 1.2	4.0 ± 0.5	1.9 ± 0.5
0.02 mg/ml	74.4 ± 2.2*	20.1 ± 2.2*	3.8 ± 0.8	1.7 ± 0.4
0.2 mg/ml	78.7 ± 2.1	17.0 ± 2.4	3.3 ± 0.7	0.9 ± 0.3
2.0 mg/ml	81.1 ± 2.3	10.8 ± 2.0	5.7 ± 0.7	2.3 ± 0.7

[a]Values represent mean ± SE derived from 22–24 mice in the deionized water group and 14–16 mice in the other groups.
*Significantly different from control at $p < 0.05$.

Sera were analyzed for selected enzymes, ions, and other marker molecules, including glucose, blood urea nitrogen, and creatinine. In all, 14 serum chemistry parameters were evaluated (Tables 10 and 11). In the control mice, glucose, cholesterol, SGOT, SGPT, and SAP were all slightly elevated compared to our historical controls[10]. The serum chemistry values altered in males which could be considered exposure-related were the 28%

TABLE 9
Hematology of Female CD-1 Mice Exposed to TCE in the Drinking Water for 90 Days[a]

Parameter	Deionized Water	TCE 0.02 mg/ml	TCE 0.2 mg/ml	TCE 2.0 mg/ml
Hematocrit (%)	41.0 ± 0.3	40.8 ± 0.5	40.8 ± 0.6	39.1 ± 0.5*
Hemoglobin (g%)	14.0 ± 0.1	13.9 ± 0.3	14.2 ± 0.2	13.1 ± 0.3*
Erythrocytes ($10^6/mm^3$)	8.36 ± 0.17	8.39 ± 0.31	8.34 ± 0.20	7.75 ± 0.19
Leukocytes ($10^3/mm^3$)	4.34 ± 0.24	5.06 ± 0.37	4.88 ± 0.23	6.42 ± 0.44*
Platelets ($10^5/mm^3$)	3.76 ± 0.13	4.21 ± 0.11*	4.12 ± 0.16	4.19 ± 0.16*
Fibrinogen (mg%)	188 ± 3	225 ± 6*	211 ± 7*	209 ± 7*
Prothrombin Time (seconds)	9.8 ± 0.1	9.5 ± 0.2	9.1 ± 0.2*	8.0 ± 0.1*
APTT[b] (seconds)	33.1 ± 1.2	33.1 ± 1.4	31.8 ± 0.8	36.5 ± 1.5

[a]Values represent mean ± SE derived from 23 mice in the deionized water group and 16 mice in the other groups.
[b]APTT = activated partial thromboplastin time.
*Significantly different from control at $p < 0.05$.

increase in cholesterol levels and the 61% increase in SAP activity in mice exposed to the highest concentration of TCE. The exposure-related alterations in the females included a 36% increase in cholesterol levels and a 63% increase in SGPT activity at the highest dose level, and SGOT and SAP increases at all dose levels (not dose dependent).

Liver glutathione levels decreased dose dependently in males exposed to the two highest concentrations of TCE by 16 and 28%

TABLE 10
Serum Chemistry and Liver Glutathione Levels of Male CD-1 Mice Exposed to TCE in the Drinking Water for 90 Days[a]

Parameter	Deionized Water	TCE 0.02 mg/ml	TCE 0.2 mg/ml	TCE 2.0 mg/ml
Calcium (mg%)	11.4 ± 0.2	12.0 ± 0.2	12.4 ± 0.2*	12.1 ± 0.2*
Phosphorus (mg%)	6.90 ± 0.20	6.91 ± 0.26	6.63 ± 0.28	6.57 ± 0.26
Sodium (mEq/L)	146 ± 1	154 ± 3*	149 ± 1	152 ± 2*
Chloride (mEq/L)	112 ± 2	111 ± 2	115 ± 5	121 ± 4*
Potassium (mEq/L)	7.49 ± .21	8.32 ± .21*	6.84 ± .21*	6.74 ± .18*
Protein (g%)	7.85 ± .23	7.77 ± .24	8.03 ± .23	8.25 ± .27
Glucose (mg%)	183 ± 10	171 ± 11	185 ± 11	207 ± 15
Cholesterol (mg%)	305 ± 15	304 ± 21	342 ± 16	392 ± 22*
BUN (mg%)	34.1 ± 1.5	29.0 ± 1.0*	31.8 ± 1.3	31.7 ± 1.0*
LDH (IU/L)	638 ± 55	589 ± 91	736 ± 45	676 ± 64
SGPT (IU/L)	62.3 ± 8.4	40.6 ± 3.4	39.9 ± 4.3*	53.1 ± 8.3
SGOT (IU/L)	116.5 ± 14.0	91.4 ± 8.6	79.0 ± 4.2*	104.2 ± 10.5
SAP (IU/L)	53.0 ± 4.2	52.2 ± 7.1	57.6 ± 8.6	85.1 ± 9.5*
Creatinine (mg%)	0.34 ± .02	0.25 ± .03*	0.31 ± .02	0.38 ± .01
Glutathione (μmol/g liver)	10.83 ± .44	9.96 ± .52	9.08 ± .25*	7.77 ± .51*

[a]Values represent mean ± SE derived from 24 mice in the deionized water group and 14–16 mice in the other groups. Glutathione levels were derived from 8 mice per group.
*Significantly different from control at $p < 0.05$.
BUN = blood urea nitrogen; LDH = lactate dehydrogenase; SGPT = serum glutamic-pyruvic transaminase; SGOT = serum glutamic-oxaloacetic transaminase; SAP = serum alkaline phosphatase

TABLE 11
Serum Chemistry and Liver Glutathione Levels of Female CD-1 Mice Exposed to TCE in the Drinking Water for 90 Days[a]

Parameter	Deionized Water	TCE 0.02 mg/ml	TCE 0.2 mg/ml	TCE 2.0 mg/ml
Calcium (mg%)	11.4 ± 0.2	12.1 ± 0.3*	11.9 ± 0.2	11.7 ± 0.2
Phosphorus (mg%)	7.17 ± 0.26	7.02 ± 0.34	7.22 ± 0.30	7.04 ± 0.26
Sodium (mEq/L)	166 ± 1	163 ± 3	162 ± 1	166 ± 2
Chloride (mEq/L)	110 ± 3	115 ± 4	116 ± 3	114 ± 3
Potassium (mEq/L)	7.66 ± .15	6.96 ± .26*	6.51 ± .26*	7.22 ± .25
Protein (g%)	7.58 ± .13	8.26 ± .25*	7.95 ± .21	8.21 ± .25*
Glucose (mg%)	159 ± 7	175 ± 7	167 ± 9	168 ± 7
Cholesterol (mg%)	219 ± 8	223 ± 12	247 ± 17	298 ± 20*
BUN (mg%)	25.0 ± 0.9	32.4 ± 1.6*	25.8 ± 1.4	29.2 ± 1.3*
LDH (IU/L)	662 ± 42	708 ± 66	768 ± 59	806 ± 52
SGPT (IU/L)	36.9 ± 2.2	49.8 ± 5.6	46.8 ± 7.0	60.2 ± 8.8*
SGOT (IU/L)	83 ± 5	117 ± 12*	107 ± 7*	107 ± 8*
SAP (IU/L)	56.8 ± 3.4	78.2 ± 4.0*	73.2 ± 5.9*	68.8 ± 4.3*
Creatinine (mg%)	0.38 ± .02	0.40 ± .02	0.40 ± .03	0.40 ± .02
Glutathione (μmol/g liver)	8.00 ± .17	8.33 ± .18	7.59 ± .22	9.07 ± .09*

[a]Values represent mean ± SE derived from 24 mice in the deionized water group and 16 mice in the other groups. Glutathione levels were derived from 8 mice per group.
*Significantly different from control at $p < 0.05$.
BUN = blood urea nitrogen; LDH = lactate dehydrogenase; SGPT = serum glutamic-pyruvic transaminase; SGOT = serum glutamic-oxaloacetic transaminase; SAP = serum alkaline phosphatase

TABLE 12
Hepatic Microsomal Activities in Male CD-1 Mice Exposed to TCE in the Drinking Water for 90 Days[a]

Parameter	Deionized Water	TCE 0.02 mg/ml	TCE 0.2 mg/ml	TCE 2.0 mg/ml
Microsomal Protein (mg/g liver)	23.8 ± 1.0	23.9 ± 1.5	25.2 ± 0.9	22.8 ± 1.1
Cytochrome P-450 (nmol/mg protein)	1.129 ± .069	1.048 ± .046	1.110 ± .072	1.030 ± .054
Cytochrome b5 (nmol/mg protein)	.392 ± .018	.389 ± .019	.424 ± .021	.387 ± .034
Aminopyrine N-demethylase (nmol/mg/min)	11.8 ± 0.7	11.5 ± 0.7	10.9 ± 0.6	10.8 ± 0.5
Aniline Hydroxylase (nmol/mg/min)	2.03 ± .12	1.72 ± .06	2.23 ± .09	2.45 ± .25

[a]Values represent mean ± SE derived from 8 mice per group.

(Table 10). Female mice exposed to the highest concentration of TCE showed a 13% elevation in glutathione levels (Table 11).

The effects of 90 day exposure to TCE upon hepatic microsomal activities are shown in Tables 12 and 13. In males, there were no perturbations of microsomal protein, cytochrome P-450 and b5 content. Microsomal enzymes that demethylate aminopyrine or hydroxylate aniline were not affected (Table 12). Changes did occur with some of these parameters in the female mice (Table 13). Cytochrome P-450 content and aniline hydroxylase activity were reduced dose dependently, while microsomal protein, cytochrome b5, and aminopyrine demethylase activity were unaffected.

TABLE 13
Hepatic Microsomal Activities in Female CD-1 Mice Exposed to TCE in the Drinking Water for 90 Days[a]

Parameter	Deionized Water	TCE 0.02 mg/ml	TCE 0.2 mg/ml	TCE 2.0 mg/ml
Microsomal Protein (mg/g liver)	22.4 ± 0.9	22.1 ± 0.6	23.8 ± 0.6	22.6 ± 0.4
Cytochrome P-450 (nmol/mg protein)	1.080 ± .029	1.014 ± .031	.978 ± .029*	.798 ± .018*
Cytochrome b5 (nmol/mg protein)	.491 ± .010	.454 ± .014	.464 ± .015	.494 ± .009
Aminopyrine N-demethylase (nmol/mg/min)	13.6 ± 0.9	12.7 ± 0.5	12.4 ± 0.5	13.0 ± 0.4
Aniline Hydroxylase (nmol/mg/min)	1.74 ± .04	1.57 ± .07	1.50 ± .08*	0.92 ± .04*

[a]Values represent mean ± SE derived from 8 mice per group.
*Significantly different from control at $p < 0.05$.

DISCUSSION

There is increased interest in the chlorinated hydrocarbons, particularly the haloalkanes and haloalkenes, since they have been identified in finished drinking water supplies[3]. With the discovery of previously unknown chemical dumps and potential contamination of drinking water supplies, there is a need for more definitive toxicological data assessing the potential hazard of these contaminants.

The LD50's for male and female mice for TCE administered by the oral route (378 mg/kg for males and 491 mg/kg for females) are slightly higher than those reported by Klassen and Plaa[5] for the i.p. route in male mice (242 mg/kg). These values are

similar to the LD50 for 1,2-dichloroethane (489 and 413 mg/kg for male and female mice, respectively)[13]. Deaths from acute administration of TCE and 1,2-dichloroethane appear to result from depression of the central nervous system. In contrast, trichloroethylene and trans-1,2-dichloroethylene are much less toxic than TCE with the LD50's for males and females for trichloroethylene being 2402 and 2443 mg/kg[14], and being 2122 and 2391 mg/kg, respectively, for trans-1,2-dichloroethylene[15].

In the 14-day range-finding study conducted on male mice, no definitive toxicity could be detected at doses of 3.8 and 38 mg/kg. Thus, in the 90-day study, concentrations in the drinking water were established to deliver 3.8, 38, and 380 mg/kg. The highest dose would represent an LD50 dose for males, if given as a bolus. In preliminary preference taste tests, mice did not avoid the chemical (data not shown); however, in the 90-day study, males, but not females, consumed less fluid when TCE was present (Table 4). This decreased fluid consumption in males may have caused a decrease in their growth rate. Based on fluid consumption, the males consumed a time-weighted average dose of 308 mg/kg/day at the highest dose level of TCE. Female mice showed no preference for deionized water over TCE-containing water, as seen in fluid consumption data and in their growth pattern.

The only significant dose-related effects due to 90 days of exposure to TCE in males was a decreased liver glutathione level and an elevation in SAP. There was also an elevation in serum cholesterol; however, since the control group showed levels higher than those usually found in our historical controls[10], the interpretation of these data is difficult. The reduction in liver glutathione and the elevation of SAP indicate an effect on the liver of males. However, there were no elevations of SGOT or SGPT in males, which would be expected if an exposure of this duration resulted in liver damage. It may be that the consump-

tion of liver glutathione protected the liver and possibly other target tissues of male mice from the effects of this chemical. This was not the case for females. Not only were glutathione levels not decreased, but they were elevated along with SGOT, SGPT, SAP, fibrinogen levels, and liver weight. There was also a decrease in cytochrome P-450 content and aniline hydroxylase activity, which provides the basis for potential synergistic effects with other drugs and chemicals.

In these studies, the lowest effect level seen in females was 0.2 mg/ml (44 mg/kg), which resulted in a reduction of cytochrome P-450 levels and aniline hydroxylase activity. The no adverse effect level was 0.02 mg/ml (3.9 mg/kg). In males, the lowest effect level was 0.2 mg/ml (46 mg/kg), which resulted in reduction of liver glutathione, and the no adverse effect level was 0.02 mg/ml (4.4 mg/kg).

TCE appears to have a short half life and little bioaccumulation considering the fact that mice given the LD50 in the drinking water daily for 90 days did not result in any deaths. Furthermore, few other toxic signs were noted, among them being loss of body weight and changes in liver parameters. This indicates that TCE may not be a serious hazard in the chronic situation and that it may be more important to monitor acute exposure to this compound.

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